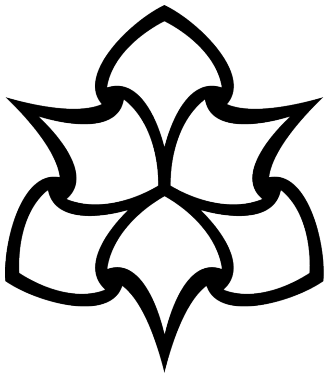

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Hitting the Jackpot – development of gas chromatography–mass spectrometry (GC–MS) and other rapid screening methods for the analysis of 18 fentanyl-derived synthetic opioids

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Abstract

In recent years, the occurrence of synthetic opioid fentanyl and its derivatives has grown significantly in forensic casework. This study presents the synthesis and analysis of 18 fentalogs, selected based on information received from local law enforcement. This study provides colorimetric tests, thin-layer chromatography (TLC) which can potentially be utilized for presumptive screening of the target compounds, as bulk powders or as trace-level adulterants. The fully validated confirmatory GC–MS method (employing SIM mode) allows the identification of the 18 derivatives, five commonly encountered controlled substances and four adulterants, within 20 minutes. The cross-validated method described herein provides a sensitive screening and quantitation method for the illicit (and potentially harmful) components at trace levels (LOD = 0.007–0.822 μg/mL and LOQ = 0.023–2.742 μg/mL respectively). Spectral data [^{1}H-NMR, ^{13}C-NMR, ^{19}F-NMR, FT-IR, and HRMS] and assignments for the synthesized reference materials are also provided in the Supplementary Information for laboratories engaged in the routine analysis of fentanyl and its derivatives.

KEYWORDS

characterization, fentalogs, fentanyl, GC-EI-MS, synthetic opioids, triage

1 | INTRODUCTION

Fentanyl (**2b**) was first patented as an analgesic in 1965 and eventually reached widespread medical use due to its very strong and fast action.[1,2] However, due to its euphoria-inducing effects resembling those of heroin (**8c**), it has also been used recreationally since the 1980s.[3,4] From 2013 onward, fentanyl abuse has grown significantly in the USA, reaching "epidemic levels".[5,6] This situation poses a serious threat for public health, not just in the USA but potentially worldwide, as minute quantities of fentanyl could potentially be enough to induce a lethal overdose. The prevalence of fentanyl analogs or fentalogs (**2a**, **2c–2k, 2n–2r**, Figure 1), some even more potent than the original, on drug markets has become a serious issue for law enforcement and healthcare providers. In the period 2013–2019, 32 new fentalogs were reported to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) – with this number growing every year.[7,8] The emergence of fentalogs represents a challenge for law enforcement, health, and harm reduction practitioners, as the reporting of extensive chemical information about analogs in academic journals cannot keep up with the speed at which those substances appear.[5,9] Though principally associated with the United States, the global significance of these synthetic opioids within forensic casework has been highlighted through a number of toxicological reports in which fentanyl-laced heroin has been implicated in fatalities in Canada and Australia.[10-12] Though fentanyl and its analogues have been principally combined with heroin (**8c**) or are being sold in combination with U-47700 as "fake Norco" (a formulation of acetaminophen and hydrocodone), it has also been detected in street samples of cocaine (**7**), and even purportedly sold as 3′,4′-methylenedioxymethamphetamine (MDMA, **3**) – which potentially may have more serious implications to wider drug using communities.[9,13-19] The development of simple, sensitive methods for the screening of fentanyl and its analogs at trace level in complex mixtures is therefore crucial for public health protection.

Previous studies have reported the analysis of fentanyl (**2b**) and its analogs (see Supplementary Information; Table S1). Sisco et al. have reported a very sensitive direct analysis in real-time mass spectrometry [DART-MS, limit of detection = 0.08–0.35 ng] and ion mobility spectrometry [IMS, limit of detection = 1.0–10.0 ng] screening methods, but neither of these techniques facilitated efficient

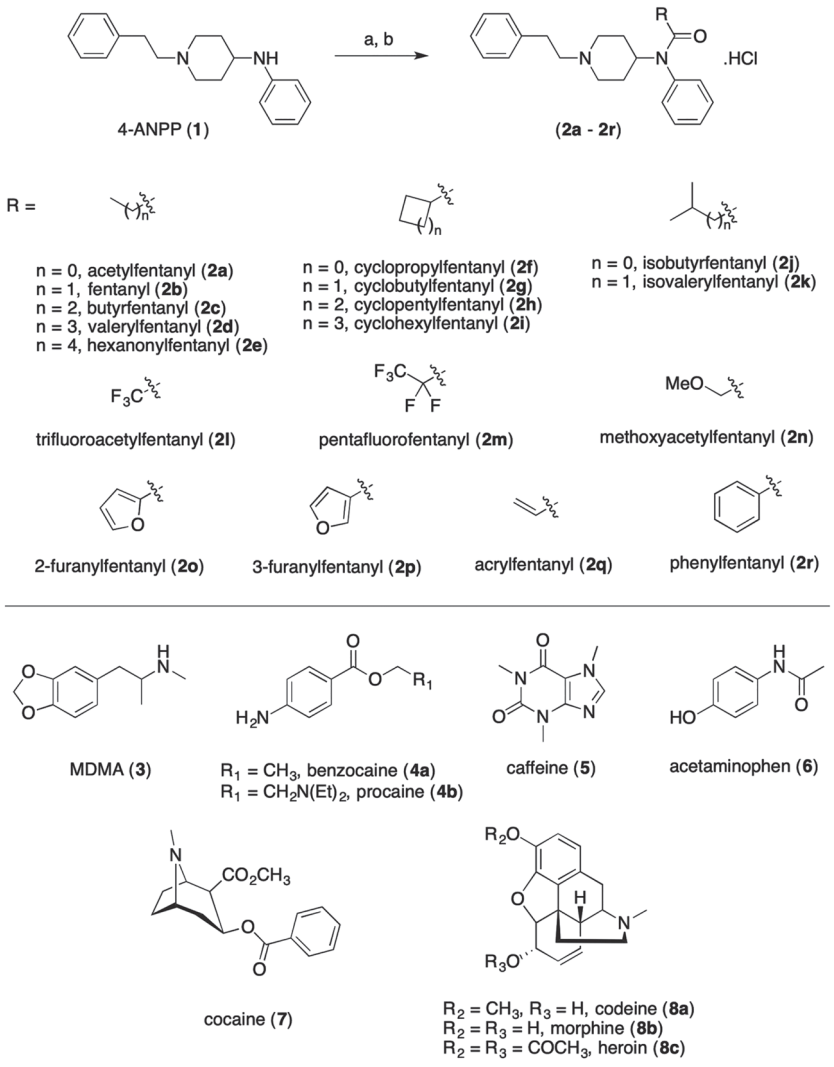

FIGURE 1 Structures of fentanyl hydrochloride (**2b**), its derivatives (**2a**, **2c–2r**), common substances of abuse and adulterants. Reagents and conditions: (A) RCOCl (2.0 eq)/$^{i}Pr_2NEt$ (2.0 eq)/CH_2Cl_2; (B) HCl (3 M in cyclopentyl methyl ether, 1.0 eq)/Et_2O or acetone (15–64% yield). See materials and methods (Section 2.1) for experimental details

separation of the 18 analogs within the study.[20] High performance liquid chromatography (HPLC) has been applied in a number of studies[21-23] including one validated method, which has been developed and utilized to quantify (**2b**) within bulk forensic samples of heroin.[22] Hyphenated techniques (LC–MS, LC-MS^2 and UPLC-MS^2) have also been applied to detect fentalogs and their metabolites in blood,[24-27] urine[25] and wastewater.[28] Although these methods are impressively quick, they were not optimized to chromatographically resolve the targeted analytes, which can lead to ion suppression when analyzing low-concentration, adulterated street samples.[29] More importantly, the published method(s) rely on equipment that is prohibitively expensive for smaller forensic laboratories, which normally rely on gas chromatography–mass spectrometry (GC–MS) as a primary method of analysis.[30] The United Nations Office on Drugs and Crime (UNODC) have recently published guidelines for the identification and analysis of (**2b**) and its analogs – primarily focused on their detection within biological samples.[31] Bravo et al., Strano-Rossi et al., and Misailidi et al. have also independently developed GC–MS methods for the determination of (**2b**),[32,33] sufentanil,[33] alfentanil,[33] 2-furanylfentanyl (**2o**)[34] and ocfentanil[34] in toxicological/post-mortem samples, however, surprisingly simple validated GC–MS methods with the ability to separate and quantify an array of fentalogs, for the routine analysis of bulk samples, both in their pure form and in the presence of other controlled substances or adulterants have not yet been reported in the literature.

Seeking to address this issue, a general GC-EI-MS screening method for 18 fentanyl derivatives is reported herein. The selection of the derivatives, including two novel examples (**2l** and **2m**) reported herein, was based on the current literature regarding prevalence (see Supplementary Information Table S2) and information provided by local law enforcement and public health officials operating within Greater Manchester, UK. This validated method allows quantification of the target compounds, in pure form or at trace level in the presence of common drugs and adulterants. Presumptive methods of detection (i.e. thin-layer chromatography and colorimetric tests) were also investigated as a potential tool for the rapid, on-site identification of those drugs. Additionally, characterization data [^{1}H-NMR, ^{13}C-NMR, ^{19}F-NMR (for compounds **2l** and **2m**), FT-IR] for the synthesized reference material are reported in the Electronic Supplementary Information and serve as additional comparative information for laboratories engaged in the routine analysis of fentalogs.

2 | MATERIALS AND METHODS

All reagents were of commercial quality (Sigma-Aldrich, Gillingham, UK or Fluorochem Limited, Hadfield, UK) and used without further purification. Solvents (Fisher Scientific, Loughborough, UK) were dried, where necessary, using standard procedures.[35] The target compounds (**2a**–**2r**) were synthesized, from 4-ANPP (**1**), using an adaptation of the method reported by Valdez et al.[2] and obtained as stable, off-white powders (> 99.5% purity by NMR and HRMS). The NMR purity was calculated using the relative concentration determination method described by Pauli et al.[36] ^{1}H-NMR (10 mg/600 μL in d_6-DMSO) and ^{13}C-NMR spectra (20 mg/600 μL in d_6-DMSO) were acquired on a JEOL JMN-ECS-400 (JEOL, Tokyo, Japan) NMR spectrometer operating at a proton resonance frequency of 400 MHz and referenced to the residual solvent peak (d_6-DMSO: ^{1}H-NMR δ = 2.50 ppm, ^{13}C-NMR δ = 39.52 ppm[37] respectively). ^{19}F-NMR spectra (10 mg/600 μL in d_6-DMSO containing 0.03% v/v trifluoroacetic acid, TFA) for compounds (**2l**, **2m**) were acquired on the same instrument and referenced to TFA (^{19}F-NMR, δ = −76.55 ppm[38]). Infrared spectra were obtained in the range 4000–400 cm^{-1} using a Thermo Scientific Nicolet iS10ATR-FTIR instrument (Thermo Scientific, Rochester, USA). High-resolution mass spectrometry (HRMS) data were obtained on an Agilent 6540 LC-QToF spectrometer in positive electrospray ionization mode. Melting points were acquired on a Stuart SMP10 digital melting point apparatus. The seven seized samples of heroin were provided by Greater Manchester Police, in accordance with Manchester Metropolitan University's Home Office license requirements and agreed procedures.

2.1 | Synthesis

The hydrochloride salts of fentanyl (**2b**) and its derivatives (**2a**, **2c–2r**) were prepared as reported by Valdez et al.[2] with the following modifications: *N*-[1-(2-phenylethyl)-4-piperidinyl]aniline (4-ANPP, **1**, 1.35 g, 4.8 mmol) was added to dichloromethane (40 mL) and was treated with diisopropylamine (1.68 mL, 9.6 mmol, 2 eq). The system was flushed with argon, the mixture cooled in an ice bath and the appropriate acyl chloride (9.6 mmol, 2 eq) added dropwise. The resulting solution was stirred at ambient temperature for 2 h. The mixture was diluted with water (50 mL) and the organic phase washed sequentially with brine (1 × 50 mL) and saturated aqueous sodium bicarbonate solution (1 × 50 mL), dried with magnesium sulfate, and concentrated in vacuo. The crude oils were purified by gravity column chromatography (SiO_2, 3:7–7:3 v/v EtOAc–hexane). The free base(s) were dissolved in either diethyl ether or acetone, and an equimolar amount of hydrogen chloride (3 M in cyclopentyl methyl ether) was added. The mixture was left to stand for 5–10 minutes and the salt isolated by filtration. The product(s) were dried in an oven (60°C, 12 h) to give white to off-white powders, which were fully characterized by ^{1}H-NMR, ^{13}C-NMR, ^{19}F-NMR (**2l**, **2m**), FTIR, HRMS, and melting point (see Supplementary Information, Table S3). Yields of products (based on 4-ANPP, after purification) were as follows: acetylfentanyl hydrochloride (**2a**, 33%); fentanyl hydrochloride (**2b**, 41%); butyrylfentanyl hydrochloride (**2c**, 39%), valerylfentanyl hydrochloride (**2d**, 30%), hexanoylfentanyl hydrochloride (**2e**, 43%); cyclopropylfentanyl hydrochloride (**2f**, 64%); cyclobutylfentanyl hydrochloride (**2g**, 64%); cyclopentylfentanyl hydrochloride (**2h**, 37%); cyclohexylfentanyl hydrochloride (**2i**, 36%); isobutyrylfentanyl hydrochloride (**2j**, 60%); isovalerylfentanyl hydrochloride (**2k**, 33%); trifluoroacetylfentanyl hydrochloride (**2l**, 38%); pentafluorofentanyl hydrochloride (**2m**, 52%); methoxyacetylfentanyl

hydrochloride (**2n**, 29%); 2-furanylfentanyl hydrochloride (**2o**, 32%), and phenylfentanyl hydrochloride (**2r**, 15%).

2.2 | Presumptive tests

Presumptive tests were carried out according to the United Nations recommended guidelines.[39,40] The following standard presumptive tests were applied in this study: (i) Marquis; (ii) Scott's; (iii) nitric acid and (iv) Eosin Y tests. The preparation of the reagents and the test procedure is detailed below. Six repetitive tests of each compound were conducted and negative control samples were used in all tests.

Marquis test: 1% Formaldehyde (37% aqueous solution) in concentrated sulfuric acid (10 mL, d = 1.86 g/mL). Each test sample (1–2 mg) was placed into a separate dimple well of a white spotting tile and 2 drops of the test reagent added. Any color change or other noticeable effect occurring immediately on addition of the reagents was noted and observations were made again after 5 min.

Scott test: 1% Cobalt (II)thiocyanate in glycerol-deionized water (1:1, 10 mL). Each test sample (1–2 mg dissolved in 1–2 drops of methanol) was placed into a separate dimple well of a white spotting tile and 2 drops of the test reagent added. Any color change or other noticeable effect occurring immediately on addition of the reagents was noted and observations were made again after 5 min.

Nitric acid test: Concentrated nitric acid (d = 1.51 g/mL). Each test sample (1–2 mg) was placed into a separate dimple well of a white spotting tile and 2 drops of the test reagent added. Any color change or other noticeable effect occurring immediately on addition of the reagents was noted and observations were made again after 5 min.

Eosin Y test: 150 μM Eosin Y (2′,4′,5′,7′-tetrabromofluorescein) in aqueous potassium phosphate buffer (pH 7). Each test sample (1–2 mg) was placed into a separate dimple well of a white spotting tile and 2 drops of the test reagent added. Any color change or other noticeable effect occurring immediately on addition of the reagents was noted and observations were made again after 5 min.

2.3 | Thin layer chromatography (TLC)

Thin layer chromatography (TLC) was carried out on aluminum-backed SiO_2 plates (Merck, Germany). The mobile phase used was dichloromethane-methanol (9:1 v/v) containing 1% triethylamine. The developed plate was viewed under UV light (254 nm) and any spots noted. The plate was sprayed with modified Dragendorff-Ludy-Tenger reagent,[41] the orange spots were marked with a pencil and the retention factor (R_f), and the relative retention factor (RR_f, with respect to fentanyl, **2b**) calculated for each analyte. Six repetitive tests of all compounds were conducted and negative control samples were used in all tests. Photographs of the TLC plates for the standards (**1**, **2a**–**2r** and **8c**) and the seven samples of suspected heroin (SS-1–SS-7) are provided in the Supplementary Information (Figure S1 and S2, respectively).

2.4 | Gas chromatography–mass spectrometry (GC–MS)

GC–MS analysis was performed using an Agilent 7890B GC and a MS5977B mass selective detector (Agilent Technologies, Wokingham, UK). The mass spectrometer was operated in the electron ionization mode at 70 eV. Separation was achieved with a capillary column (HP5 MS, 30 m Å~ 0.25 mm i.d. 0.25 μm) with helium as the carrier gas at a constant flow rate of 1.2 mL/min. The following oven temperature program was used: 175–235°C at 30°C/min, hold 7 min, 235–270°C at 30°C/min, hold 7.5 min, 270–290°C at 30°C/min, hold 2 min, for a total run time of 20.33 min. A 2 μL aliquot of the samples was injected with a split ratio of 50:1. The injector and the GC interface temperatures were both maintained at 280°C respectively. The MS source and quadrupole temperatures were set at 230°C and 150°C. Mass spectra were obtained in full scan mode (50–550 amu). All samples (qualitative analysis) were prepared as 1 mg/mL solutions in methanol with no derivatization and analyzed individually and in combination with five commonly encountered controlled substances (MDMA, **3**; cocaine, **7**; codeine, **8a**; morphine, **8b**, and heroin, **8c**) and four adulterants (benzocaine, **4a**; procaine, **4b**; caffeine, **5**, and acetaminophen [paracetamol], **6**). Eicosane (0.5 mg/mL) was used as an internal standard and each sample was injected six times.

2.5 | Calibration standards

Ten mg of each analyte (**2a**–**2r**), *N*-[1-(2-phenylethyl)-4-piperidinyl] aniline (4-ANPP, **1**), **3**, **4a**, **4b**, **5**, **6**, **7**, **8a**–**8c** was weighed accurately into a 10.0 mL clear glass class A volumetric flask and diluted to volume with methanol to give a solution containing all components at 1 mg/mL. This solution was then further diluted with methanol and 100 μL of eicosane (50 μg/mL in methanol) added (in each case) to give calibration standards containing 2.5 μg/mL, 5.0 μg/mL, 10.0 μg/mL, 20.0 μg/mL, and 25.0 μg/mL of each analyte and the internal standard at 5.0 μg/mL.

2.6 | GC–MS method validation

GC–MS method validation was performed using an Agilent 7890B GC and a MS5977B mass selective detector (Agilent Technologies, Wokingham, UK) employing the parameters detailed in Section (2.4). Mass spectra were obtained under selected ion monitoring (SIM) mode, using three specific fragment ions for each analyte (Supplementary Information, Table S3 and S5). The GC–MS method was validated in accordance with the ICH guidelines[42] using the

following parameters: linearity, accuracy, precision (repeatability), limit of detection (LOD), and limit of quantification (LOQ). Linearity, precision: six replicate injections of the calibration standards were performed and the data analyzed under the same conditions. The %RSD was calculated for each replicate test sample. Accuracy (percentage recovery study): determined from spiked samples prepared in triplicate at three levels over a range of 80–120% of the target concentration (15 μg/mL). The percentage recovery and %RSD were calculated for each of the replicate samples. Repeatability (intraday precision) and intermediate precision (interday precision): determined from six replicate injections of a spiked sample (10 μg/mL), analysed on two consecutive days. The percentage purity and %RSD were calculated for each of the replicate samples. Limits of detection and quantification: six replicate injections of the calibration standards were performed and the data analyzed under the same conditions. The limits of detection and quantification were determined based on the signal-to-noise (S/N) ratio, where a signal-to-noise ratio of 3:1 and 10:1 was used to calculate the LOD and LOQ respectively.[42] Signal-to-noise ratios were measured over six injections in the lower end of the concentration range (2.5 μg/mL for most analytes; 5.0 μg/mL for morphine) using the auto-root-mean-squared (Auto-RMS) algorithm from the Agilent MassHunter Qualitative Analysis software.

2.7 | Test solutions (qualitative GC–MS analysis)

The seven samples of suspected heroin were obtained from Greater Manchester Police (Manchester, UK; July 2018) and used without further purification. The individual samples were homogenized and arbitrarily labelled, SS-1–SS-7, prior to analysis. Each test substance was weighed accurately (10.0 mg) into a 10.0 mL clear glass class A volumetric flask, diluted to volume with methanol and filtered. This solution was then further diluted (8:2, 1.0 mL) with 100 μL methanol and 100 μL eicosane (50 μg/mL in methanol) added (in each case) to give a test solution containing ca. 15 μg/mL of the sample and the internal standard at 5.0 μg/mL. The test solutions were injected in triplicate and mass spectra were obtained in full scan mode (50–550 amu).

2.8 | Test solutions (quantitative GC–MS analysis)

Each test substance (SS-1–SS-7) was weighed accurately (12.5 mg) into a 5.0 mL clear glass class A volumetric flask, diluted to volume with methanol and then filtered. This solution was then further diluted (8:2, 1.0 mL) with 100 μL methanol and 100 μL eicosane (50 μg/mL in methanol) added (in each case) to give a test solution containing ca. 15 μg/mL of the sample and the internal standard at 5.0 μg/mL. The test solutions were injected in triplicate. Quantification of the primary components: caffeine (**5**), acetaminophen (**6**), and heroin (**8c**) was determined in full scan mode (50–550 amu), whereas fentanyl (**2b**) or its analogs (**2a**, **2c**–**2r**) was determined using selected ion monitoring (SIM) mode, using three specific fragment ions for each analyte (see Supplementary Information, Tables S3 and S5).

3 | RESULTS AND DISCUSSION

3.1 | Synthesis

Samples of 18 fentanyl derivatives (see Supplementary Information; Table S3) were prepared as their corresponding hydrochloride salts. The selection of the derivatives and inclusion of trifluoroacetylfentanyl (**2l**) and pentafluorofentanyl (**2m**) was based on the current literature (See Supplementary Information, Table S2) and information provided by local law enforcement and public health officials operating in Greater Manchester, UK. The synthesis of the target compounds was achieved using a modification of the method reported by Valdez et al.[2] from *N*-[1-(2-phenylethyl)-4-piperidinyl]aniline (4-ANPP, **1**) and the corresponding acyl chloride, in 15–64% overall yield, as stable, white to off-white powders (Figure 1). The hydrochloride salts were determined to be soluble (10 mg/mL) in deionized water, methanol, and dimethylsulfoxide and the purity of all samples was confirmed to be > 99.5% (by NMR and HRMS) in all cases. The spectral data [^{1}H-NMR, ^{13}C-NMR, ^{19}F-NMR (for compounds **2l** and **2m**), FT-IR] with assignments for the synthesized reference material are provided in the Supplementary Information (Figure S3–S59) for comparison.

3.2 | Thin layer chromatography

Suzuki et al.[46] have reported the retention factors (R_f) for 25 fentalogs including (**2a**–**2c**) and (**2j**). However, under the conditions reported [SiO_2, chloroform-benzene-methanol (10:2:1 v/v/v)] the authors were unable to fully discriminate these analogs. When thin layer chromatography [SiO_2, dichloromethane-methanol (9:1 v/v) containing 1% triethylamine] was carried out on the 18 derivatives (**2a**–**2r**), the spots produced by each analog gave identical colors (orange) when viewed with modified Dragendorff-Ludy-Tenger reagent. The TLC data for each compound, including their retention factor (R_f) and relative retention factor (RR_f, with respect to fentanyl, **2b**) and photographs of the plates are presented in the Supplementary Information (Table S3 and Figure S1). Examination of the R_f values (six replicates) demonstrated separation of 14 of the compounds based upon this measure, particularly the cycloalkyl series (**2f**–**2i**, R_f = 1.02, 1.05, 1.09, and 1.13, respectively). Separation was less clear-cut for the other isomeric derivatives: valerylfentanyl (**2d**, R_f = 1.06) vs. isovalerylfentanyl (**2k**, R_f = 1.09) and 2-furanylfentanyl (**2o**, R_f = 1.04) vs. 3-furanylfentanyl (**2p**, R_f = 1.09). In the case of butyrylfentanyl (**2c**) and isobutyrylfentanyl (**2j**) the two derivatives co-eluted, which is analogous to observations reported by Suzuki et al.[46] Though full resolution of the 18 analogs from **1** (R_f = 0.36) and heroin (**8c**, R_f = 0.43) was achieved under these conditions, it was difficult to discriminate

between all 18 analogs by TLC alone and therefore further analysis was required.

3.3 | Presumptive tests

Kangas et al. have recently disclosed the presumptive testing of fentanyl (**2b**) in both its pure form or in the presence of either cocaine (**7**) or hydrocodone using commercially available NIK-A (Marquis) and NIK-G (modified Scott's) kits and Eosin Y (2′,4′,5′,7′-tetrabromofluorescein) dissolved in either phosphate (pH 7) or acetate (pH 5) buffer.[43] Though this study was able to easily discriminate between the three analytes and demonstrate that Eosin Y could be employed in the rapid detection of fentanyl (**2b**), its scope in terms of detecting other analogs was not explored. The following standard presumptive color tests were carried out according to the United Nations recommended guidelines[39,40] in this study: (i) Marquis test; (ii) Scott's test; (iii) Nitric acid test and (iv) Eosin Y test. The results indicated that all the derivatives (**2a**–**2r**), containing a tertiary amine, gave a positive reaction with the Marquis, Scott's, and Eosin Y reagents (see Supplementary Information; Table S4). These results are in agreement with Kangas' observations and infer that Eosin Y has potential for the detection of a wide range of fentanyl derivatives when used in combination with the other two reagent tests. In the case of the Scott's reagent, which is employed in the screening of cocaine, the colored products are believed to result from the coordination of the tertiary amines to the pink Co (II) octahedral complex affording the blue Co (II) tetrahedral complex.[40,44] The colored products observed in the Marquis test may be rationalized by the reaction of the drug molecules with sulfuric acid in a mechanism analogous to that of the reaction of MDMA.[40] The concentrated nitric acid test gave negative reactions with the majority of derivatives except for 2-furanylfentanyl (**2o**) and 3-furanylfentanyl (**2p**) – which produced a pale yellow color after 5 min – allowing differentiation between them and 4-ANPP (**1**), MDMA (**3**), acetaminophen (**5**), and the morphine-based opiates (**8a**–**8c**). The positive response of (**2o**) and (**2p**) was not readily explained but may have resulted from electrophilic attack on the furan ring, potentially facilitating discrimination between these derivatives and other fentalogs if a secondary screen was required.

The observed color changes (Supplementary Information, Table S4) indicated that Eosin Y reagent – currently unavailable as a commercial test kit – could provide a simple and rapid test for these materials when used in combination with Marquis and Scott's test. Though other common adulterants and controlled drugs also formed colored products with the Marquis (**3**, **8a**, **8b**, and **8c**) and Eosin Y (**1**, **3**, **4b**, **5**, **6**, **7**, and **8a**–**8c**) reagent and/or blue Co (II) tetrahedral complexes (**4b**, **7**, and **8c**) with Scott's reagent, the observed colors were significantly different with the Eosin Y to allow for their discrimination. The recommendation of this study is that three presumptive tests (Eosin Y, Marquis, and Scott's) could be employed, to discriminate between controlled drugs and/or adulterants and fentanyl-derived synthetic opioids, with the nitric acid test used as a secondary screen in cases where the results are not clear cut.

3.4 | Gas chromatography–mass spectrometry

The qualitative GC–MS method (ca. 20 min) used required an extremely straightforward solvation of the samples in methanol (0.1 mg/mL) followed by direct injection into the instrument. No derivatization step was required. In most cases, the fentanyl derivatives were resolved from each other and five commonly encountered

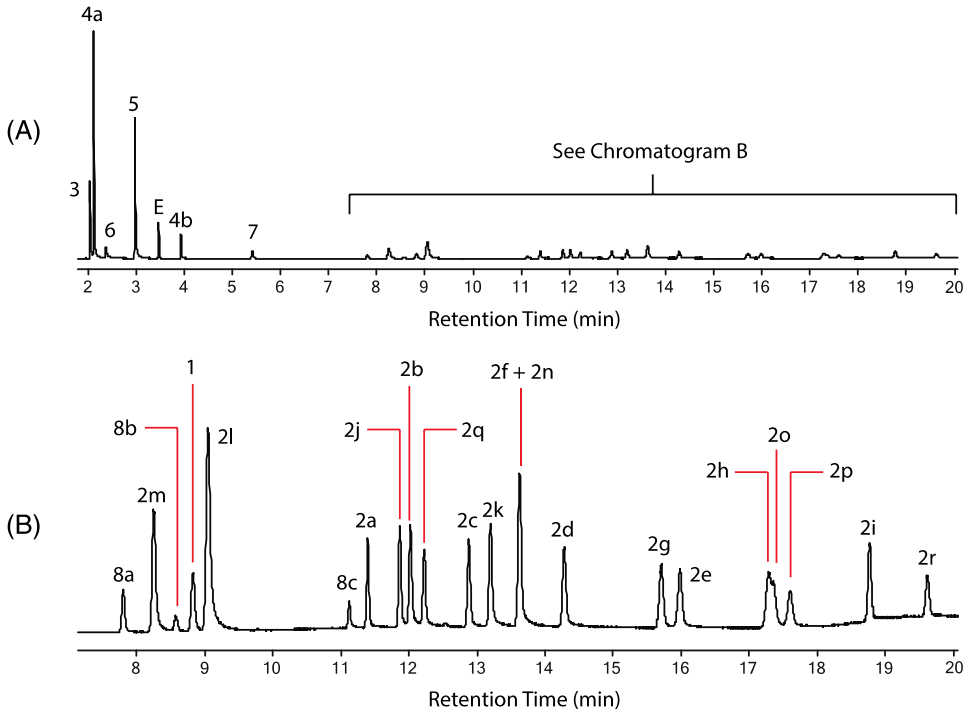

FIGURE 2 Exemplar chromatogram demonstrating separation of 18 fentanyl derivatives (**2a**–**2r**), controlled substances and relevant adulterants: 3,4-methylenedioxymethamphetamine (MDMA, **3**), benzocaine (**4a**), acetaminophen (**6**), caffeine (**5**), eicosane (internal standard, E), procaine (**4b**), cocaine (**7**), codeine (**8a**), morphine (**8b**), *N*-[1-(2-phenylethyl)-4-piperidinyl]aniline (4-ANPP, **1**), and heroin (**8c**) [underlined compounds are common adulterants]. See materials and methods (Section 2.4) for experimental details [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Summary of GC–MS validation data (selective ion monitoring mode) for the quantification of fentanyl (**2b**), its derivatives (**2a**, **2c**–**2r**), controlled substances (**1**, **3**, **7**, **8a**–**8c**) and relevant/common adulterants (**4a**, **4b**, **5**, and **6**). NB. t_R (eicosane) = 3.47 min. See Figure 2 for representative chromatogram

											Precision (% RSD, n = 6)					
	SIM ions (for quantification)	t_R (min)	RRT[a]	R_s[b]	A_s[c]	N[d] (plates)	H[e] (x10^{-4} mm)	r^2	LOD[ah] (μg/mL)	LOQ[ai] (μg/mL)	2.5 μg/mL	5 μg/mL	10 μg/mL	15 μg/mL	20 μg/mL	25 μg/mL
3	135.1, 77.0, 58.0	2.04	0.17	-	2.4	136219	2.20	0.996[f]	0.023	0.007	3.6	2.1	1.3	2.5	1.4	3.2
4a	165.1, 137.0, 120.1	2.11	0.18	3.9	1.6	228879	1.31	0.992[g]	0.023	0.007	6.7	2.9	1.9	2.1	0.9	3.0
6	151.0, 109.0, 80.0	2.36	0.20	9.3	5.6	93224	3.22	0.992[h]	0.793	0.238	5.8	6.4	4.0	3.5	1.7	3.4
5	194.1, 109.0, 82.0	3.0	0.25	18.2	2.4	200019	1.50	0.994[i]	0.045	0.014	6.8	4.3	1.6	1.8	1.4	2.3
4b	120.1, 99.1, 86.1	3.91	0.33	14	1.2	196150	1.53	0.999[j]	0.046	0.014	1.5	1.6	1.7	0.9	1.8	1.6
7	303.0, 182.0, 82.0	5.37	0.45	30.3	1.4	140356	2.14	0.999[k]	0.030	0.009	2.2	1.2	2.0	0.8	1.7	1.0
8a	299.1, 229.1, 162.1	7.75	0.65	33.7	1.0	136584	2.20	0.998[l]	0.073	0.022	1.7	1.6	2.0	0.8	1.9	1.8
2 m	335.1, 292.0, 239.0	8.20	0.68	4.8	1.0	113948	2.63	0.999[m]	0.025	0.007	1.6	1.1	2.2	1.0	1.8	1.7
8b	285.1, 215.1, 162.1	8.52	0.71	3.3	1.0	135096	2.22	0.999[n]	2.742	0.822	-[aj]	6.9	3.8	3.3	2.7	2.0
1	189.0, 146.1, 93.0	8.77	0.73	2.7	1.3	119168	2.52	0.999[o]	0.090	0.027	1.2	1.3	2.4	1.1	1.9	1.9
2 l	285.1, 242.0, 189.1	8.99	0.75	2.0	1.6	105886	2.83	0.999[p]	0.049	0.015	1.1	0.9	2.0	1.1	1.8	1.5
8c	369.2, 327.2, 268.1	11.09	0.92	24.5	1.0	515458	0.58	0.998[q]	0.236	0.071	2.3	1.6	2.9	0.7	1.9	2.5
2a	231.1, 189.1, 146.0	11.36	0.95	4.5	1.0	540884	0.56	0.997[r]	0.026	0.008	0.5	1.2	2.1	1.4	2.0	2.6
2j	259.1, 189.1, 146.0	11.84	0.99	7.3	1.4	510511	0.59	0.997[s]	0.058	0.017	0.7	0.9	2.1	1.2	2.3	2.3
2b	245.1, 189.1, 146.0	11.99	1.00	2.3	1.2	460776	0.65	0.998[t]	0.034	0.010	0.5	1.0	2.2	1.4	2.2	2.3
2q	243.1, 200.0, 189.1	12.19	1.02	2.8	1.4	541711	0.55	0.997[u]	0.044	0.013	1.1	1.1	2.4	1.5	2.1	2.5
2c	259.1, 189.1, 146.0	12.85	1.07	8.9	0.9	418336	0.72	0.997[v]	0.047	0.014	0.7	1.1	2.4	1.5	2.3	2.4
2 k	273.1, 189.1, 146.0	13.16	1.10	3.8	1.2	393893	0.76	0.997[w]	0.044	0.013	1.1	0.7	2.2	1.5	2.3	2.3
2f	257.1, 189.1, 146.0	13.60	1.13	4.8	1.5	343508	0.87	0.998[x]	0.082	0.025	0.8	1.4	2.4	1.3	2.1	2.2
2n	261.1, 218.0, 158.0	13.60	1.13	4.8	1.2	343508	0.87	0.998[y]	0.043	0.013	1.2	1.3	2.1	1.4	2.2	2.5
2d	273.1, 189.1, 146.0	14.25	1.19	7.1	1.2	377429	0.80	0.997[z]	0.052	0.016	0.8	1.1	2.3	1.3	2.1	2.5
2 g	271.1, 189.1, 146.0	15.68	1.31	14	1.0	322327	0.93	0.997[aa]	0.077	0.023	1.1	1.1	2.5	1.2	2.3	2.5
2e	287.2, 189.1, 146.0	15.95	1.33	2.5	1.1	333544	0.90	0.997[ab]	0.038	0.011	0.4	1.0	2.2	1.3	2.2	2.3
2 h	285.1, 189.1, 146.0	17.25	1.44	9.9	1.0	271035	1.11	0.997[ac]	0.138	0.042	1.0	0.3	2.4	1.2	2.2	2.5
2o	283.1, 240.0, 95.0	17.31	1.44	0.5	1.1	273246	1.10	0.997[ad]	0.413	0.124	3.2	1.1	4.5	1.4	2.7	2.8
2p	283.1, 240.0, 95.0	17.57	1.47	2.4	1.0	281061	1.07	0.998[ae]	0.415	0.125	4.5	1.8	2.2	1.3	2.3	4.0
2i	299.1, 189.1, 146.0	18.77	1.61	12.4	1.3	249699	1.20	0.997[af]	0.051	0.015	1.1	0.8	2.4	1.3	2.2	2.5
2r	293.1, 250.0, 105.0	19.62	1.74	9.7	1.1	247424	1.21	0.997[ag]	0.092	0.028	0.9	1.2	2.5	1.1	2.3	2.6

[a]Key: Relative retention time (with respect to fentanyl, **2b**);
[b]Resolution;
[c]Asymmetry (or tailing) factor;

[d]Number of theoretical plates;
[e]Height of a theoretical plate;
[f]y = 0.3731x-0.1005;
[g]y = 0.5935x-0.6021;
[h]y = 0.1037x-0.1519;
[i]y = 0.3539x-0.2284;
[j]y = 0.1587x + 0.1199;
[k]y = 0.0742x + 0.0602;
[l]y = 0.0488x + 0.0531;
[m]y = 0.1322x + 0.1305;
[n]y = 0.0129x-0.0235;
[o]y = 0.0904x + 0.0401;
[p]y = 0.1642x + 0.1149;
[q]y = 0.0208x + 0.0295;
[r]y = 0.0926x + 0.1140;
[s]y = 0.0846x + 0.1060;
[t]y = 0.0930x + 0.1058;
[u]y = 0.0924x + 0.1122;
[v]y = 0.0926x + 0.1140;
[w]y = 0.0936x + 0.1139;
[x]y = 0.0963x + 0.1098;
[y]y = 0.1004x + 0.0963;
[z]y = 0.0911x + 0.1138;
[aa]y = 0.0924x + 0.1198;
[ab]y = 0.0943x + 0.1206;
[ac]y = 0.0866x + 0.1103;
[ad]y = 0.0904x + 0.1040;
[ae]y = 0.0863x + 0.1045;
[af]y = 0.0951x + 0.1214;
[ag]y = 0.0852x + 0.0906;
[ah]Limit of detection [calculated using a signal-to-noise (S/N) ratio of 3:1];
[ai]Limit of quantification [calculated using a signal-to-noise (S/N) ratio of 10:1];
[aj]Not determined as concentration is below the LOQ for morphine (**8b**)

controlled substances (cocaine, codeine, heroin, MDMA, morphine) and four adulterants (acetaminophen [paracetamol], benzocaine, caffeine, and procaine). An exemplar chromatogram is presented in Figure 2. The use of GC–MS also facilitated the visualization of the mass spectral data for each individual compound, and these are presented in Figure 1. In the case of the two co-eluting derivatives (**2f** and **2n**, t_R = 13.6 min), differentiation was achieved through direct comparison of the mass spectral data. Though both derivatives underwent primary α,β-cleavage of the phenethylamine moiety, the resulting base peaks (**2f**, m/z = 257 vs. **2n**, m/z = 261) and subsequent fragmentation patterns were significantly different.[45] In the case of (**2f**) secondary cleavage of the base peak (m/z = 257) via scission of either the piperidine ring or the amide group gave rise to two fragments (m/z = 214 and m/z = 189, respectively), which underwent further dissociation affording the fragment (m/z = 146) common to many fentanyl-type opioids (Figure 2).[46] The electron ionization (EI) mass spectrum for methoxyacetylfentanyl (**2n**) included fragment ions at m/z = 261 (base peak), 218, 190, 158, 91, and 45 ($C_2H_5O^+$), which correspond to the data obtained by Jannetto et al.[45] Clear discrimination of the co-eluting derivatives was achieved using selected ion monitoring (SIM), employing three distinct ions for each analyte (**2f**, m/z = 257.1, 189.1, and 146.0; **2n**, m/z = 261.1, 218.0, and 158.0). The two partially resolved analytes (**2h**, t_R = 17.2 min and **2o**, t_R = 17.3 min) were also discriminated using SIM mode (**2h**, m/z = 285.1, 189.1, and 146.0 vs. **2o**, m/z = 283.1, 240.0, and 95.0) and both underwent EI fragmentation analogous to other fentanyl-derived opioids, affording fragment ions which were in agreement with the literature (Figure 2).[46]

A number of groups have reported utilizing HPLC[21-23] or GC–MS[32-34] for the toxicological screening of fentanyl (**2b**) and its derivatives within bulk powders and biological matrices with a recent report disclosing the development of a high performance liquid chromatographic method employing amperometric detection (HPLC-AD).[22] Despite being able to detect [LOD = 0.45–2.93 μg/mL] and quantify [LOQ = 1.49–9.76 μg/mL] 11 fentalogs (**2a**–**2d**, **2f**–**2h**, **2j**–**2k**, **2n**, and **2r**), the method utilized a specialized sensing platform and lacked selectivity for heroin (**8c**) vs. cocaine (**7**) and the two common adulterants (caffeine, **5** and acetaminophen, **6**), normally found in seized bulk samples. Interestingly, though GC–MS methods are routinely employed by forensic laboratories for both the identification and quantification of drugs of abuse, no validated quantitative GC–MS methods that provide simple general screening and quantification of the components in bulk samples have, to date, been reported. The quantitative GC–MS method (SIM mode), using three ions specific to each analyte (Supplementary Information, Table S3), was developed and validated in accordance with the ICH guidelines.[42] To facilitate accurate identification of the compounds present within seized samples the ion ratios (relative to the base peak) for the three ions specific to each analyte were determined. The accuracy (%RSD) of the ion ratios was calculated from three injections of each analyte and showed 0.3–7.7% variation between replicates (Supplementary Information, Table S5). Calibration standards were prepared and all 18 substituted fentalogs demonstrated a linear response (r^2 = 0.997–0.999) over a 2.5–25.0 μg/mL range with satisfactory repeatability (RSD = 0.3–4.5%, n = 6). Due to the level of sensitivity required for the detection of the fentanyl analogs within bulk samples the limits of detection (LOD) and quantification (LOQ) were determined for both scan mode and selective ion monitoring mode (see Supplementary Information, Table S6). In scan mode the LOD/LOQs were determined to be ~100× less sensitive and as such the selective ion monitoring mode was deemed more suitable for this application. The limits of detection and quantification for the analytes (in bulk samples) were determined (for SIM mode), based on the signal to noise (S/N) ratio, as being 0.008–0.125 and 0.025–0.415 μg/mL, respectively, which is ~50× more sensitive than the recently published HPLC-AD method. The method was also suitable for the detection and quantification of the five commonly encountered controlled substances [cocaine, codeine, heroin, MDMA, and morphine] and four adulterants [acetaminophen (paracetamol), benzocaine, caffeine, and procaine], demonstrating linear response (r^2 = 0.992–0.999) over the same concentration range with reasonable repeatability (RSD = 0.7–6.9%, n = 6). The limits of detection and quantification

TABLE 2 Qualitative and quantitative analysis of seized samples (SS-1–SS-7) obtained from Greater Manchester Police (Manchester, UK, July 2018)

Sample no.	Weight (g)	Compounds detected
SS-1	0.07	Acetaminophen (**6**), 5.9 ± 0.5% w/w; Caffeine (**5**), 3.69 ± 0.06% w/w Heroin (**8c**), 53.1 ± 0.8% w/w; fentanyl (**2b**), 6.29 ± 0.01% w/w
SS-2	0.12	Acetaminophen (**6**), 26.8 ± 1.5% w/w; Caffeine (**5**), 14.8 ± 0.8% w/w Heroin (**8c**), 20.5 ± 0.8% w/w; fentanyl (**2b**)[a], 0.288 ± 0.008% w/w Minor components: Diacetyl-*p*-aminophenol (**9**), 6-mono-acetylmorphine (**10**)
SS-3	0.11	Acetaminophen (**6**), 27.1 ± 0.6% w/w; Caffeine (**5**), 16.9 ± 0.7% w/w Heroin (**8c**), 25.5 ± 0.7% w/w Minor components: Diacetyl-*p*-aminophenol (**9**), 6-mono-acetylmorphine (**10**)
SS-4	0.73	Acetaminophen (**6**), 28.6 ± 2.0% w/w; Caffeine (**5**), 20.5 ± 1.3% w/w Heroin (**8c**), 17.8 ± 0.4% w/w Minor components: Diacetyl-*p*-aminophenol (**9**), 6-mono-acetylmorphine (**10**)
SS-5	1.04	Heroin (**8c**), 82.9 ± 2.7% w/w Minor component: 6-mono-acetylmorphine (**10**)
SS-6	0.95	Heroin (**8c**), 74.7 ± 1.6% w/w Minor component: 6-mono-acetylmorphine (**10**)
SS-7	0.15	Heroin (**8c**), 82.2 ± 3.1% w/w Minor component: 6-mono-acetylmorphine (**10**)

[a]Key: Component only detected in SIM mode.

were determined for the controlled substances and adulterants, and found to be 0.007–0.822 and 0.023–2.742 μg/mL, respectively (Table 1). The accuracy (percentage recovery study) of the assay was determined from spiked samples prepared in triplicate at three levels over a range of 80–120% of the target concentration (15 μg/mL). The repeatability (%RSD) of the method and the percentage recovery (% assay) for each of the three replicate samples demonstrated good recoveries (100 ± 3%) for all 18 analytes within the desired concentration range (Supplementary Information, Table S7). The precision (inter- and intraday precision) was calculated from six replicate injections of a spiked sample (10 μg/mL) representing 100% of the test concentration, analysed on two consecutive days (Supplementary Information, Table S8). In most cases the inter- and intraday precision was within acceptable limits (100 ± 2%), except for **4b** (95.9% after 24 h) and **8c** (95.0% after 24 h), which may result from hydrolysis of the analytes. The GC–MS method and its validation parameters are summarized in Table 1 and Tables S5–S8 (Supplementary Information) were deemed suitable for the routine analysis of the seven street samples.

3.5 | Forensic application

Seven bulk samples (SS-1–SS-7) were obtained from Greater Manchester Police (Manchester, UK, December 2018), weighed between 0.07–1.04 g and were suspected to contain heroin (**8c**). The samples varied in color from light brown to dark beige, which potentially indicates them originating from either Southwest Asia or Columbia.[47]

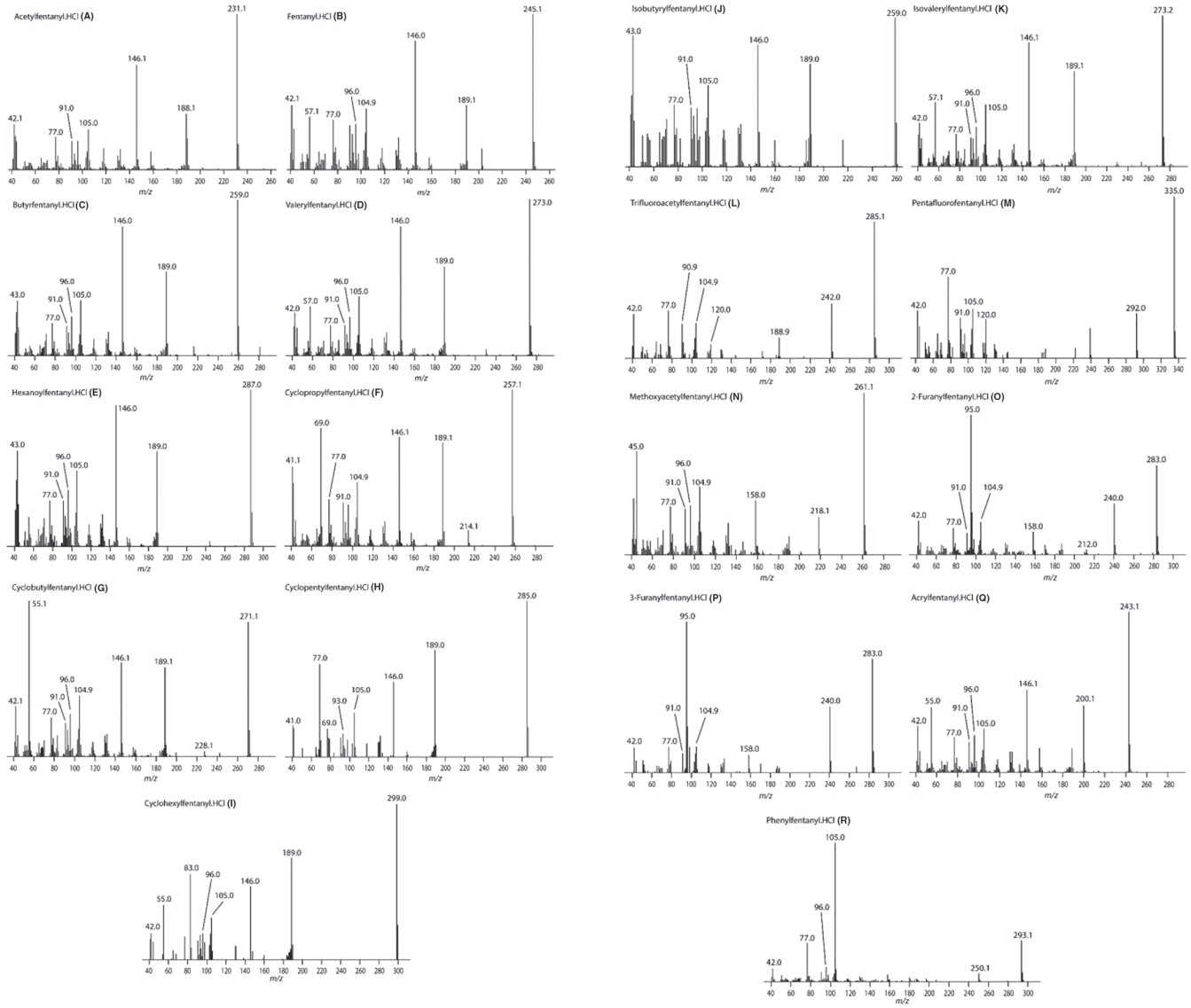

FIGURE 3 EI-MS spectra of acetylfentanyl hydrochloride (**2a**); fentanyl hydrochloride (**2b**); butyrylfentanyl hydrochloride (**2c**), valerylfentanyl hydrochloride (**2d**), hexanoylfentanyl hydrochloride (**2e**); cyclopropylfentanyl hydrochloride (**2f**); cyclobutylfentanyl hydrochloride (**2g**); cyclopentylfentanyl hydrochloride (**2h**) and cyclohexylfentanyl hydrochloride (**2i**); isobutyrylfentanyl hydrochloride (**2j**); isovalerylfentanyl hydrochloride (**2k**); trifluoroacetylfentanyl hydrochloride (**2l**); pentafluorofentanyl hydrochloride (**2m**); methoxyacetylfentanyl hydrochloride (**2n**); 2-furanylfentanyl hydrochloride (**2o**), and phenylfentanyl hydrochloride (**2r**)

Preliminary presumptive tests were carried out according to the procedures reported herein. The seven samples (SS-1–SS-7) gave positive reactions with the Marquis (brown-purple) test potentially indicating the presence of heroin (**8c**) or another opioid, but the inherent color of the sample matrix made positive identification difficult. Only one of the samples (SS-1) gave a positive reaction with Eosin Y (deep pink) potentially indicating the presence of fentanyl (**2b**) or a structural analog. However, at low concentrations, a color change indicating a positive response may have been obscured by the inherent color of the matrix. The seven samples gave inconclusive results with both Scott's reagent and concentrated nitric acid and no inference could be made on the substances that may have been present – demonstrating the limitation of colorimetric testing for samples of this nature. Thin layer chromatographic (TLC) analysis of the seven samples was performed and comparison of the samples with the reference materials confirmed the presence of heroin (**8c**, R_f = 0.22) in all seven samples. All seven samples (SS-1–SS-7) showed significant levels of adulteration, however, the principal component was determined to be heroin (**8c**, R_f = 0.22) and one sample (SS-1) potentially indicated the presence of fentanyl (**2b**, R_f = 0.46) (see Supplementary Information, Figure S2). Preliminary FT-IR analysis indicated the presence of heroin (ester C=O bands at ~1756 and ~1727 cm^{-1}) in all seven samples (see Supplementary Information, Figure S60–S66). Detailed examination of the spectral bands (amide C=O band at ~1644 cm^{-1}) potentially indicated the presence of fentanyl (**2b**) in only one of the seven samples (SS-1) (see Supplementary Information, Figure S60).

Qualitative GC–MS analysis (scan mode) corroborated the presumptive tests and confirmed the presence of heroin (**8c**, t_R = 11.1 min, m/z = 369.2, 327.2 [base peak], and 268.1) in all seven samples, with three (SS-5–SS-7) containing heroin as the single component (see Supplementary Information, Figure S67). The remaining samples (SS-1–SS-4), were determined to contain heroin, caffeine (**5**, t_R = 3.0 min, m/z = 194.1, 109.0, 82.0) and acetaminophen (**6**, t_R = 2.4 min, m/z = 151.0, 109.0, 80.0) as the primary adulterants. Three samples

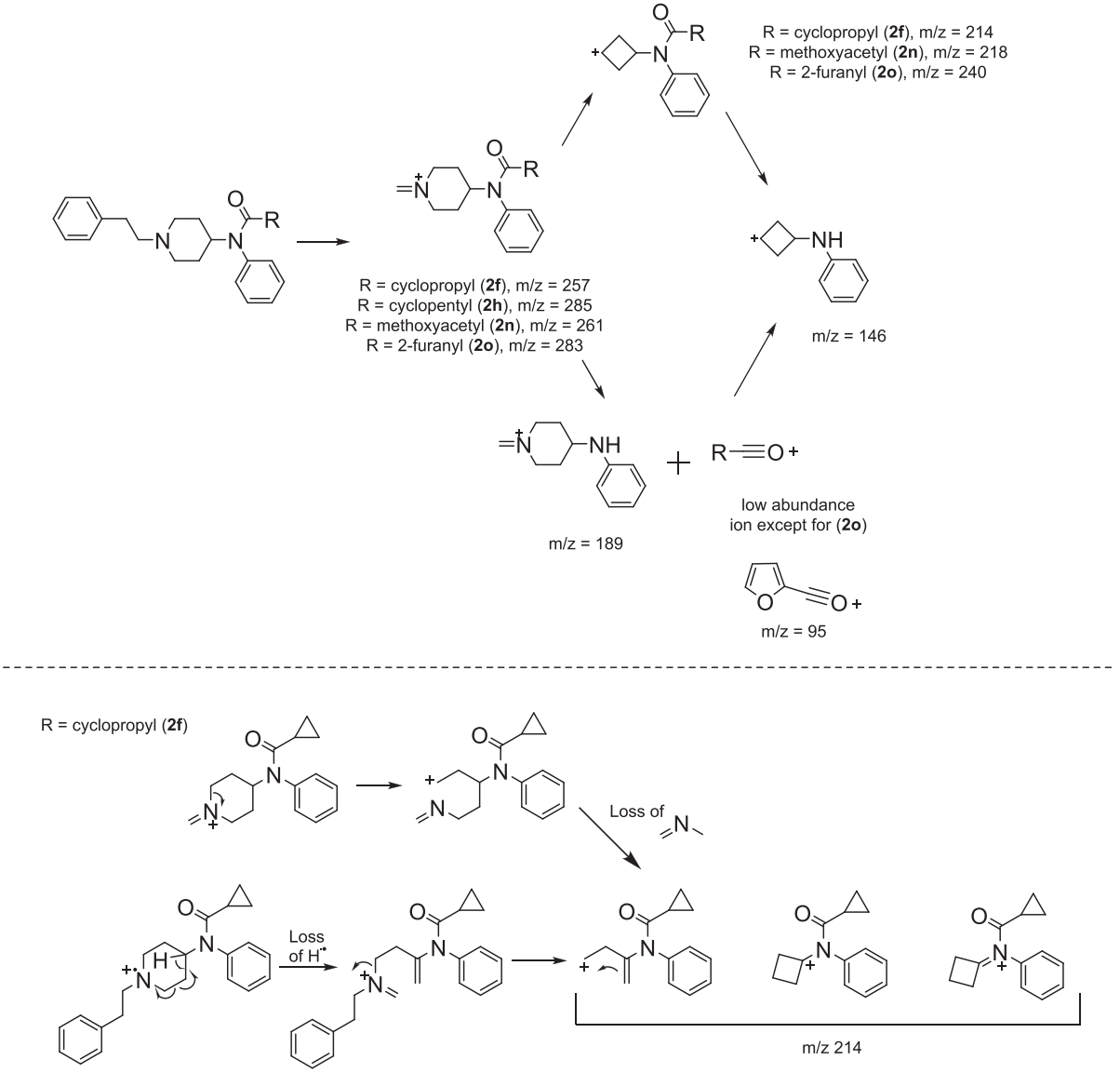

FIGURE 4 Proposed fragmentation patterns for fentanyl derivatives (**2f**, **2h**, **2n**, and **2o**)

(SS-2–SS-4) contained additional (minor) peaks, which were determined to be N,O-diacetylaminophenol (DAAP, t_R = 2.6 min, m/z = 193.0, 151.0, and 109.0) and six (SS-2–SS-7) contained 6-mono-acetylmorphine (6-MAM, t_R = 9.9 min, m/z = 327.1, 268.1, and 215.0). N,O-diacetylaminophenol has been observed to form via transacetylation between acetaminophen (**6**) and *o*-acetylsalicylic acid (aspirin) combinations that have been stored for prolonged periods[48] and may have arisen, in these samples, from a similar interaction between (**6**) and (**8c**). The presence of hydrolysis product 6-MAM is postulated to arise if heroin samples are stored in damp conditions over a period of time.[49] One sample (SS-1, Supplementary Information Figure S67A) indicated the presence of fentanyl (**2b**, t_R = 12.0 min, m/z = 245.1, 189.1, 146.0), which agreed with the preliminary tests carried out on this sample.

With substantial evidence supporting a GC–MS approach for quantifying fentalogs in heroin street samples, the applicability of the optimized quantification method was tested. The samples were reanalyzed (in triplicate) using the validated GC–MS method at a concentration of 15 μg/mL. Quantification of the primary components (caffeine (**5**), acetaminophen (**6**), and heroin (**8c**)) was performed in full scan mode (50–550 amu), whereas analysis of fentanyl (**2b**) or its analogs (**2a**, **2c–2r**) was performed in SIM mode, using three specific fragment ions for each analyte (Table 1). The quantitative GC–MS results confirmed that all seven samples contained heroin (t_R = 11.1 min, **8c**) at levels ranging between 17.8–82.9% w/w, with the lower purity samples (SS-1–SS4) containing significant levels of the commonly used diluents caffeine (**5**, 3.7–20.5% w/w) and acetaminophen (**6**, 5.9–28.6% w/w) (Table 5).[50]

As preliminary analysis of SS-1 (0.07 g) (Figure 3A) indicated the presence of fentanyl (**2b**, t_R = 11.9 min), it was necessary to quantify it and the other components, using our validated GC–MS (SIM) method. Selective ion monitoring using the characteristic ions (m/z = 245.1, 189.1, 146.0) (Figure 3E) indicated that the sample contained 6.29 ± 0.01% w/w (n = 3) of (**2b**), equating to 4.403 ± 0.007 mg within the bulk sample (Figure 3B). Interestingly, though preliminary testing and GC–MS analysis obtained in full scan-mode (Figure 3C) did not indicate the presence of any fentanyl derivatives within sample SS-2 (0.12 g), selective ion monitoring (SIM) (Figure 3D) revealed that the sample did indeed contain (**2b**) at a level of 0.288 ± 0.008% w/w (n = 3) equating to 0.35 ± 0.01 mg within the bulk sample. Relative ion intensities for (**2b**) were within the tolerance windows prescribed by the World Anti-Doping Agency guidelines when compared with the pure reference material (see Supplementary Information, Table S5) further confirmed our assertion of the presence of fentanyl (**2b**) within the two samples (SS-1 and SS-2).[51] It is important to note that due to the small sample size (n = 7), the results presented herein may not truly reflect the typical prevalence of heroin samples that contain fentanyl nationally, however, these results

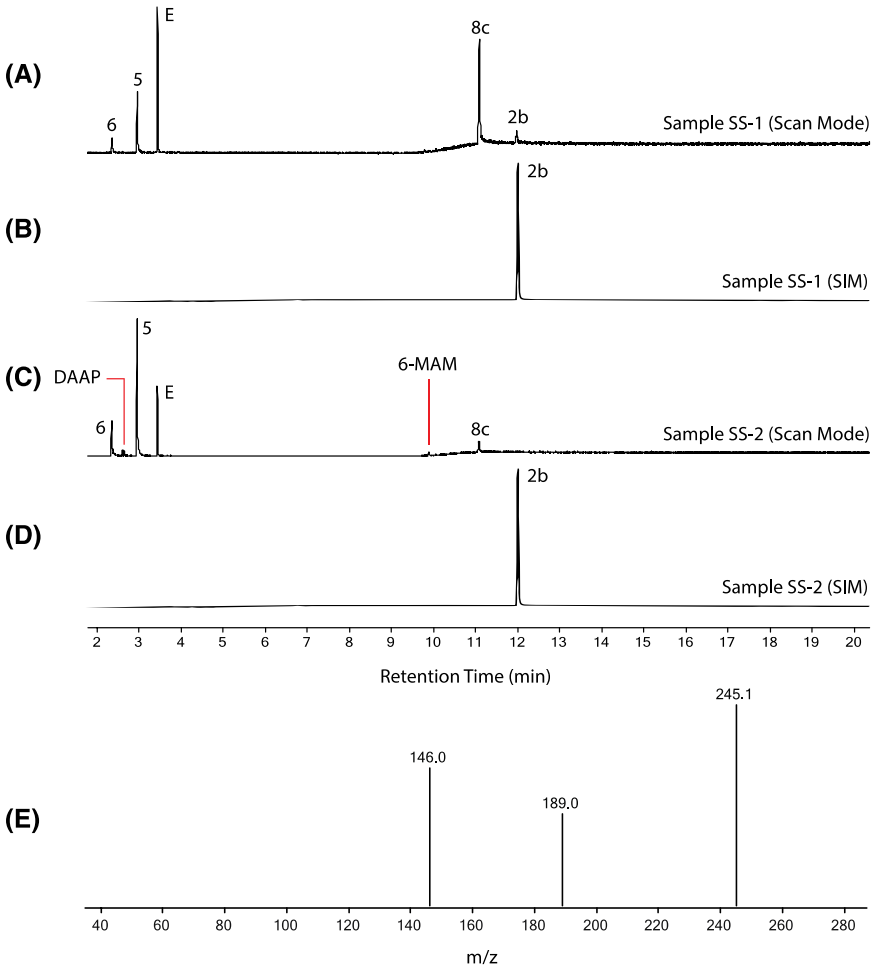

FIGURE 5 Comparison of qualitative GC–MS analysis, of seized heroin bulk samples (SS-1 and SS-2, 0.1 mg/mL in methanol) using full scan and selective ion monitoring modes: (A) GC chromatogram (full scan mode) for SS-1; (B) GC chromatogram (selective ion monitoring mode; m/z = 245.1, 189.1, 146.0) for SS-1; (C) GC chromatogram (full scan mode) for SS-2 (C) GC chromatogram (selective ion monitoring mode; m/z = 245.1, 189.1, 146.0) for SS-2; (D) SIM spectrum of peak (t_R = 12.0 min) corresponding to fentanyl (**2b**) [Colour figure can be viewed at wileyonlinelibrary.com]

demonstrate that the 20 minute GC–MS method, employing selective ion monitoring described herein is potentially suitable for the routine screening of suspect samples, which may contain fentanyl (or its derivatives) at trace levels.

4 | CONCLUSIONS

We have presented the synthesis of 18 fentalog reference materials, including two novel derivatives (**2l** and **2m**), selected based on information received by local public health officials operating in the Greater Manchester region and cross-validated the presumptive and confirmatory methods presented herein with seven samples obtained from local law enforcement. Colorimetric tests and thin layer chromatography provided a quick, presumptive detection of these compounds – however, the complex nature and matrix effects associated with adulterated samples potentially limit their application. The fully validated GC–MS method (employing SIM mode) allowed the separation and identification of all 18 fentanyls, five commonly encountered controlled substances [cocaine, codeine, heroin, MDMA, and morphine] and four adulterants [acetaminophen (paracetamol), benzocaine, caffeine, and procaine] within 20 minutes. When applied to seized samples, the validated method allowed sensitive screening and quantitative analysis of the illicit (and potentially harmful) ingredients at trace levels. Additionally, characterization data [^{1}H-NMR, ^{13}C-NMR, ^{19}F-NMR (for compounds **2l** and **2m**), FT-IR and HRMS] for the synthesized reference materials are reported in the Electronic Supplementary Information and serve as additional comparative information for laboratories engaged in the routine analysis of existing and novel fentalogs.

COMPLIANCE WITH ETHICAL STANDARDS

This study did not involve research on human participants or animals.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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